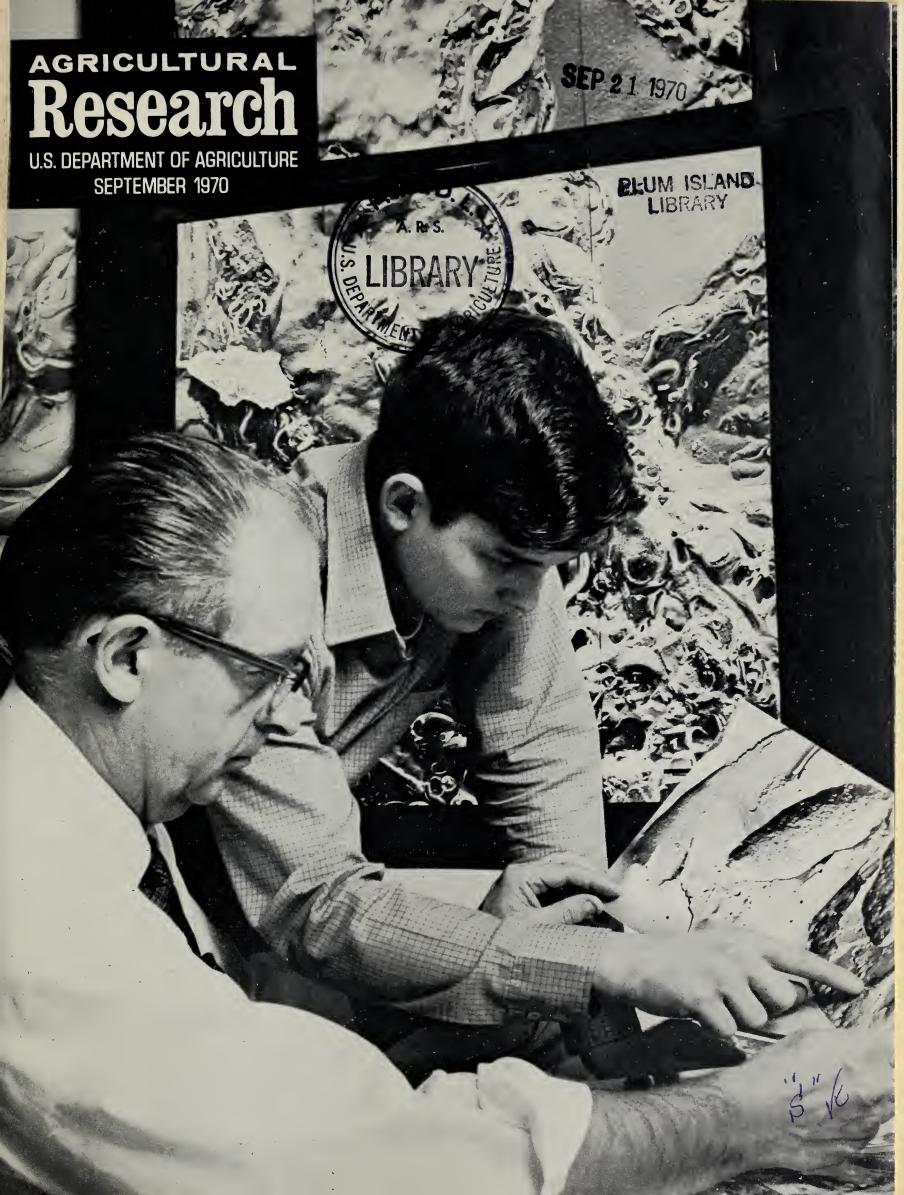
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# AGRICULTURAL RESEARCH

September 1970/Vol. 19, No. 3

### Threads of Life

All living things are delicately and interdependently linked in what is popularly called the web of life. Man too is an inseparable part of this web. But man's activities render the threads of this web vulnerable, not only at the site of application but often in seemingly remote and unrelated parts of the environment. In manipulating any part of the environment it behooves the wise man to understand the ecological workings of all the parts.

Some ecological answers will come from scientists who are patiently exploring biotic mechanisms of an intricately interwoven grazing community at the Pawnee site of ARS' 15,000-acre Central Plains Experimental Range near Nunn, Colo. This work is part of the International Biological Program, a worldwide effort involving 54 countries and scientists of many disciplines.

Once the realm of the buffalo, the Pawnee site today largely encompasses research on the production and consumption of plants in that short-grass prairie region. Scientists there study the diet of every animal that eats and is eaten. This is no small task since Pawnee is the home of some 3,000 species of insects alone.

Scientists are also studying the consequences of removing a single species from the ecosystem, the coyote who preys on forage-eating mice, for example. Special techniques are used as required. Transmitters are attached to jack rabbits, for instance, to trace their feeding ranges and measure their lifespans. Thus livestockmen who already know how much forage their ranges produce and their herds consume, will also know how much is eaten by range insects, birds, and mammals. This should lead to practices that foster beneficial species and increase forage production.

But the Pawnee studies transcend the merely economic. In this open-air lab, scientists are learning the essential nature of the prairie and how this complex habitat maintains itself. A sound knowledge of ecosystems—gained by description, analysis, and interpretation of events and processes in nature—is at the heart of environmental research. Such research becomes increasingly vital with the threat of pollution. Without natural ecosystems research, it would be difficult if not impossible to predict the consequences of pollution or devise ways to control it. Armed with this knowledge, we can help nature heal abused environments.

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**COVER:** Dr. Steere and Mr. Moseley review another set of photomicrographs of freeze-etched specimens (270A83-11).

Editor: R. P. Kaniuka Managing Editor: E. H. Davis

### Contributors to this issue:

V. R. Bourdette, V. M. Dryden,

M. C. Guilford, C. E. Herron,

L. W. Lindemer, W. W. Martin,

M. F. Tennant, D. M. Webb,

M. E. Vanderhoof

AGRICULTURAL RESEARCH is published monthly by the Agricultural Research Service (ARS), United States Department of Agriculture, Washington, D.C. 20250. Printing has been approved by the Bureau of the Budget, June 1967. Yearly subscription rate is \$1.50 in the United States and countries of the Postal Union, \$2.00 in other countries. Single copies are 15 cents each. Subscription orders should be sent to Superintendent of Documents, Government Printing Office, Washington, D.C. 20402. Use of commercial names and brands is for identification only and does not imply endorsement or approval by USDA or ARS. Information in this periodical is public property and may be reprinted without permission. Mention of the source will be appreciated but is not required.

Clifford M. Hardin, Secretary U.S. Department of Agriculture

G. W. Irving, Jr., Administrator Agricultural Research Service



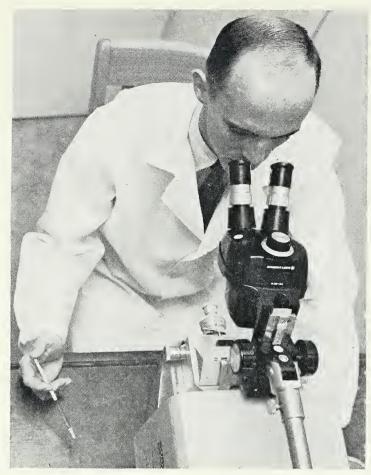
Dr. Zimmer compares electron micrographs of diseaseresistant and nondiseaseresistant safflower seedlings (PN-1891).

3

## CCB's: A Key to Genetic Resistance?

WHY DOES ONE PLANT resist disease while a slightly different variety sickens and dies? The explanation science gives is "genetic resistance." Genetic resistance simply means that a plant inherits something that makes it resistant. But what is the "something" and how it works is still a major riddle.

ARS plant pathologist David E. Zimmer and John P. Schaelling, a botany student at Utah State University, Logan, may have found a piece of the riddle, known as CCB's, (crystal-containing bodies). CCB's, since their dis-





Left: Mr. Schaelling making final adjustments of electron microscope before examining a safflower specimen (PN-1892). Above: CCB's are the crystals in the "twill-hatched" area enclosed within membrane bag (PN-1893).

covery in the last decade, have intrigued scientists, who offered many theories about their possible functions.

The very simplicity of the CCB's—tiny membrane bags of crystals—almost belies their important role in disease resistance. CCB's exist in a wide variety of plants, and their crystalline pattern varies from species to species.

Dr. Zimmer, working with rust-resistant safflower seedlings, noticed large numbers of CCB's in the cells where the seedling rust-resistant factor is believed to be located. In contrast, the cells of a rust-susceptible variety contained fewer CCB's.

Directing his efforts toward the resistant seedlings. Dr. Zimmer exposed them to rust fungus. Twelve days later, he examined the tissues of the infected plants. The cells which previously contained larger numbers of CCB's were now devoid of them. Moreover, Dr. Zimmer found significant changes in the haustoria of the fungus and the plant cells they had invaded. When a fungus invades a host cell, its

haustoria, which are tiny rootlike projections, penetrate into the host cells where they absorb the cell nutrients needed by the fungus. As the fungus grows and invades more and more cells, eventually severe damage to the plant or even death results. In his study with resistant plants, Dr. Zimmer found that portions of both the haustoria and the invaded cells had deteriorated. But when rust-susceptible seedlings were exposed to the fungus and their tissues examined, both the haustoria and the plant cells remained alive.

Dr. Zimmer believes that the greater number of CCB's found in rust-resistant seedlings, their location in the region of resistance, and their disappearance when the cells are parasitized with the rust fungus reflects some aspect of the disease-resistance mechanism.

CCB's may be storage sites of crystallized enzymes, speculates Dr. Zimmer, comparable in function to similar structures in animals, called lysosomes. Animal lysosomes are crystallized

tallized enzymes also enclosed in a membrane. Their function is to hasten the destruction of cells in their normal degenerative process.

According to Dr. Zimmer, the disappearance of the CCB's in the resistant safflower seedlings implies that they disintegrated, releasing their crystallized substances, which in turn started cell degeneration. This led to the starvation and degeneration of the fungal haustoria, which then deprived the fungal body of its life-sustaining nourishment. Thus the plant resisted disease.

CCB type of resistance may be a long-term type, rendering safflower seedlings immune to any new races of rust that might develop and the resistance is inheritable through a dominant gene.

Not only may CCB's be involved in disease resistance in some plants, says Dr. Zimmer, but they may in time with more extensive research provide scientists with an answer—at least in part—to the mechanism of disease resistance.

## sorghum bonanza

Sorghum Breeders and farmers heretofore limited to sorghum varieties that flower under comparatively long daylengths such as those in the milo, kafir, and feterita groups, will soon share in a genetic bonanza.

Under a Rockefeller-sponsored program in India and, more recently, an ARS-sponsored Public Law 480 grant, an extensive reservoir of sorghum stock, called the IS Collection, has been established by Indian scientists. The scientists assembled, characterized, and classified nearly 12,000 stocks from 44 countries.

Agronomist George F. Sprague, ARS sponsoring scientist for the Public Law 480 grant, says the importance of an adequate collection in sorghum breeding cannot be overemphasized. The rapid replacement of primitive varieties with new types poses a threat to the survival of many genotypes that are products of long periods of natural and human selection. They may well be our most valuable sources of insect and disease resistance, and their loss could be irreparable.

Because sorghum is a tropical crop and flowering is influenced by daylength, many potentially valuable varieties do not flower under U.S. conditions. To offset this, an elite group of sorghums from the IS Collection is being used in the U.S. sorghum conversion program currently underway at the Federal Experiment Station, Mayaguez, Puerto Rico, and at Texas Agricultural Experiment Station, Lub-

bock. There. ARS and Texas geneticists are crossing exotic types with early dwarf varieties to recover desirable exotic characteristics and combine them with heights and maturities needed in the United States.

From this conversion process, an estimated 100 new genetic lines will be released to U.S. breeders during 1970, and approximately 1,000 elite items will eventually become available.

Dr. Sprague, stationed at Beltsville, Md., points out that the development and maintenance of a comprehensive collection involving any crop is a continuing activity. This is particularly true for the IS Collection in which a sizable segment was accumulated by solicitation rather than by direct collection. Thus, some areas of the world are still inadequately represented. Ethiopia is one of these. To offset this, ARS and the Rockefeller Foundation in a joint venture, made

collections there in 1967. Over 1,500 items were obtained and added to the IS Collection for evaluation by ARS and Indian scientists.

The Indian work with the IS Collection has helped in differentiating discrete, or distinct. from overlapping varieties and in adding to the growing accumulation of genetic data needed by sorghum breeders.

The Indians found, for example, the presence of a genetic mechanism, previously unreported in sorghum, that prevents gene transfer between certain hybrids by producing a lethal effect such as leaf decay.

Geneticist B. R. Murty was the principal investigator for the Public Law 480 project awarded to the Indian Agricultural Institute, New Delhi. He also served as coordinator of the All India Coordinated Sorghum Project, Hyderabad, an organizational outgrowth of an earlier Rockefeller-sponsored maize project in India.

Sorghum plants in the IS Collection comprise a wide range of head types (PN-1894). Photo was taken at the Hyderabad Station assigned by the Andhra Pradesh Agricultural University to the All India Coordinated Sorghum Project.



# feeding the beef heifer...



## ..during pregnancy

CATTLEMEN ARE PRONE to heated debates when the subject turns to feeding first-calf heifers. Each seems to have his own answers as to the way and how of feeding. Calf birth weight, calving difficulty, and post partum reproduction of the heifer are all matters of general concern.

To help resolve these problems, ARS physiologist Robert A. Bellows, studied two groups of first-calf heifers at Miles City, Mont. Sixty-two Angus-Hereford crossbred heifers were bred artificially to the same Angus bull. Ninety days before calving, they were placed in two groups—one fed at a high level (group H) and the other at a low level (group L). Group H re-

ceived 20 pounds of hay and 5 pounds of grain per day, while group L received only 15 pounds of hay.

Though the heifers entered the experiment at about the same weight, group H heifers weighed 100 pounds more than those in the other group before and after calving.

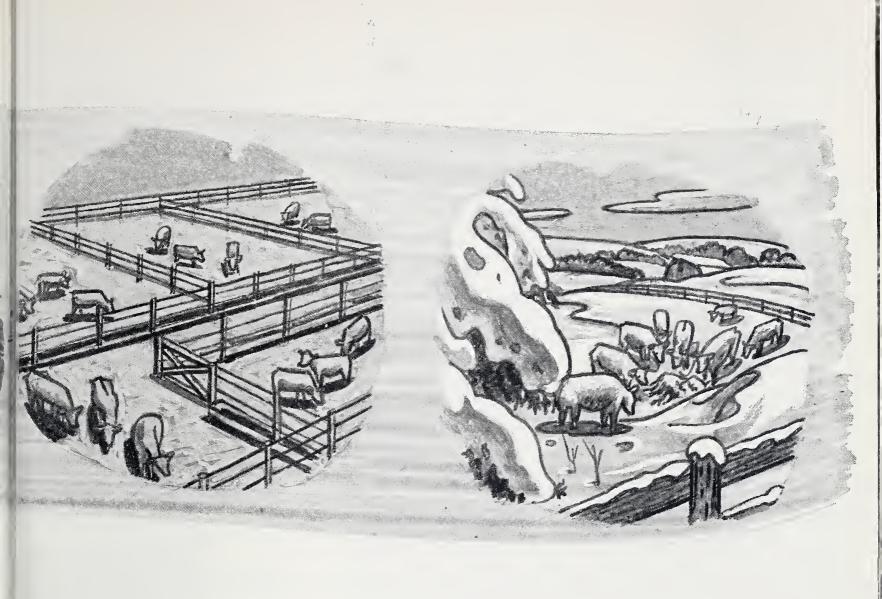
Calving difficulty was the same for both groups but Dr. Bellows warns that excessive feeding resulting in fat heifers can cause severe calving problems. Problems that may have stemmed from the high-level diet were avoided in group H by providing adequate nutrition without overfeeding.

Calves from group H heifers weighed only 4 pounds more at birth

and continued to weigh more while on the cow. When weaned, they averaged 14 pounds heavier than the calves from group L heifers.

The most striking effect of gestation feed levels, however, was on the number of cows in heat before the next breeding season. In group H, 97 percent of the cows came into heat before the regular breeding season, whereas only 68 percent of those in group L did so. Breeding at that time means an earlier calving date.

Dr. Bellows has shown that adequate nutrition of first-calf heifers, though perhaps slightly more costly at first, is the most profitable in the long run.



# ..during her first winter

THE FIRST WINTER after a heifer is weaned may be the most important season of her life. Nutrition during that time can condition her to be a high or low lifetime producer.

Poor winter feeding is false economy. ARS physiologist Robert A. Bellows, at Miles City, Mont., found that a heifer probably will not conceive early in the breeding season if she is not in good condition. Heifers in good condition, on the other hand, usually conceive early in their breeding season and continue to do so during their subsequent productive years.

Dr. Bellows studied the effects of

winter weight gains on 89 crossbred Angus-Hereford heifers. Winter gains in three groups were held at 0.5, 1.0, and 1.5 pounds per day by adjusting feed levels. After the winter feeding study, all heifers were pastured together for the summer.

Marked differences were noted in heifers at breeding time. The high-conditioned group reached puberty at an average age of 388 days as compared with 434 days for low-conditioned heifers and 412 days for the middle group.

To be good producers, heifers must conceive early in the breeding season. Dr. Bellows found that 33 percent of the high-conditioned heifers were in heat before the breeding season and 17 percent during the breeding season. This meant a possibility of a 100-percent calf crop.

On the other hand, only 7 percent of the low-conditioned heifers were in heat before and 73 percent during the breeding season. The other 20 percent did not come into heat until after the breeding season had ended. This meant only the chance of an 80-percent calf crop. All of the high-conditioned heifers were bred, but only 80 percent were bred in the low-conditioned group.

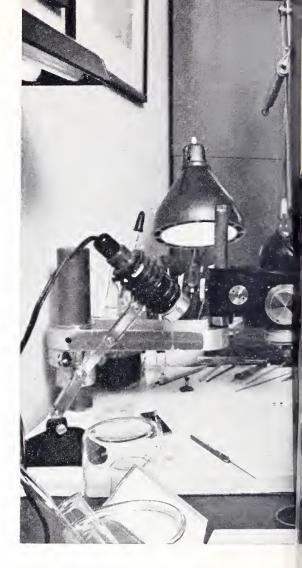
During the fall following the breeding season, 87 percent of the high-conditioned heifers were pregnant while only 50 percent of the low-conditioned heifers were—a big difference in profit when the calves arrive.

Dr. Bellows concluded that a heifer's chances of being a productive animal are very much lessened if she is not in condition to conceive early in the breeding season.



Top: Technician Michael Moseley loads holders containing frozen specimens into a vacuum chamber for fracturing (270A83-13). Right: Fractured specimens are removed from the new holder (270A83-16). Top Center: Dr. Steere places holder containing frozen specimen into special cap that can handle 8 specimens at a time (270A 80-21).





## New Perspec

N EW REFINEMENTS in the equipment used to freeze-etch specimens promise more versatility and broader capability for biological studies with electron microscopes.

The improved technique allows researchers to better study the fine details of cell membranes, including freeze damage to cells. It should permit visual studies of the development of viruses in living cells.

Freeze etching is a method that permits electron microscopic observations and photographs of biological specimens in lifelike three dimensional aspects AGR. RES. Apr. 1969, p. 10).

Originator of the technique, ARS botanist Russell L. Steere at Beltsville, Md., now has modified the equipment so that both parts of frozen-fractured specimens can be re-





Left: Specimen is quickly frozen by dipping it into a solution of Freon 22 which is surrounded by liquid nitrogen (270A80-22). Below: Dr. Steere examines fractured samples with electron microscope before photographing them (270A82-15).



## ves in Freeze Etching

tained and replicated for studies with the electron microscope. As many as 10 individual specimens can be prepared every half hour. This is 30 to 60 times as many replicas as with other freeze-etching units.

Formerly, frozen specimens were clamped to a small plate in the freeze-etching module where part of the specimen would be fractured away with a knife-edged probe. While this was an improvement over other techniques, only one part of a particular specimen could be studied since the other part was lost in the fracturing process. Dr. Steere's latest modification retains both parts, thereby opening new avenues of study.

Already, the new technique and equipment are proving their worth. For example, researchers have heretofore believed that membranes of

specimens fractured only in one plane. Dr. Steere's experiments with chicken blood cells reveal that in a single preparation, fracturing can occur at the outside surface, at the inside (cytoplasmic) surface, or the unit membranes can split down the middle.

The key feature of the modification is a newly designed specimen holder. It consists of a pair of miniature cups that are hinged on one side so that they can be closed to form a cylindrical chamber.

In tissue studies, a fragment of tissue with a total length slightly shorter than the combined depth of both cups is inserted into one cup. The other cup is then swung into place to enclose the specimen, surrounded by liquid, in the chamber.

For cell suspensions, both cups are

filled with a thick slurry and then closed.

In both cases, the entire holder is dropped into liquid Freon 22 and rapidly frozen. Then, the closed cups are clamped to a platform within the freeze-etch module. The temperature is lowered to -196° C. and a strong vacuum obtained. Fracturing is accomplished by forcing the cups apart. The etching process then follows regular procedures except that in the final mounting great care is taken to match the two parts of each specimen so that opposed surfaces can be seen and photographed through the electron microscope.

In the future experiments, Dr. Steere intends to find out if, like the chicken blood cells he's already studied, membranes of other cells also fracture in all three planes.



Atmospheric pressure and temperature are easily controlled in these special insect cages. Gordon Pearman sets up new test while Dr. Jay records temperature readings of tests in progress (570A 338-27).

Man feeds compressed  $CO_2$  into grain storage bin. In this test, bin's oxygen level was cut to 7 percent while that of  $CO_2$  was raised to 60 percent (570A343-22).



# INSECTS

S TORED-GRAIN PESTS, which are becoming increasingly resistant to available control measures, are unable to survive experimental carbon dioxide ( $CO_2$ ) treatments.

CO<sub>2</sub>, a gas naturally present in small amounts in the air, destroys insects without leaving residues and is not hazardous to handlers. ARS entomologist Edward G. Jay killed 99 percent of the maize weevils and Angoumois grain moths infesting corn in a 30,000-bushel elevator at Charleston, S.C., in a 4-day experimental treatment with CO<sub>2</sub>.

Dr. Jay released the CO<sub>2</sub> into the elevator from a tank, creating a storage atmosphere of 61 percent CO<sub>2</sub>, 8 percent oxygen, and 31 percent nitrogen. Uneven distribution of the CO<sub>2</sub> in bins was a potential drawback, as this gas is half again as heavy as air, and concentrations were lowest at the top. However, an automatic monitoring device sampled CO<sub>2</sub> concentrations at the top of the elevator and released additional CO<sub>2</sub> when the gas in the elevator fell below the level for which the monitor was set, obviating the need for manual monitoring.

CO<sub>2</sub> treatments are similar to con-

ventional fumigation procedures, which are intended to prevent damage by incipient infestations rather than to provide continuous protection to grain. The CO<sub>2</sub> continued to prove its worth 2 months after treatment of the grain, when the test ended. At that time, Dr. Jay found that insect damage to corn treated with CO<sub>2</sub> was 96 percent less than that of untreated corn.

Because of adverse conditions, the CO<sub>2</sub> treatment cost about 1½ cents per bushel. Under more favorable conditions, costs would be about ½ cent per bushel-comparable to conventional fumigation costs. The higher costs of the experimental treatment reflected the need for more CO2 than would be required in warm weather or to treat corn containing less foreign matter. Debris. which accounted for about 25 percent of the test corn, in some samples, interfered with efficient penetration of the gas. Also, the temperature averaged about 70° F.; at higher temperatures, insects respire more rapidly, and take in more CO<sub>2</sub>; thus, less of the gas would be required in warmer weather.

In laboratory tests all life stages of lesser grain borers died within 4 days when exposed to CO<sub>2</sub> at a concentration of 60 percent. Similar experiments have also been made with nitrogen gas. However, the CO<sub>2</sub> worked faster and killed more insects. Nitrogen treatments did not kill all the insects developing inside grain kernels during a 4-day exposure. Various concentrations of CO<sub>2</sub> killed the insects, but a 60-percent level provided the most satisfactory control.

Oats, peanuts, and wheat have been successfully treated with  $CO_2$  in other field tests. Further tests must be made before the  $CO_2$  treatments are ready for commercial application.

## Shaping APPLE TREES with HORMONES

THE GROWTH REGULATORS, cytokinins and gibberellins, when applied to dormant buds of apple trees nearly double shoot growth and produce branches with the desired wide crotch angles.

The first branches on an apple tree usually become the primary scaffold limbs, and limb position in a mature tree represents the greatest difference between a bearing and a nonbearing tree. A mature bearing tree has horizontal fruitful limbs whereas the limbs of a nonbearing tree are upright and vegetative. Horizontal limbs can also aid in harvesting. Limbs with narrow crotch angles tend to break and are susceptible to more winter damage and disease problems.

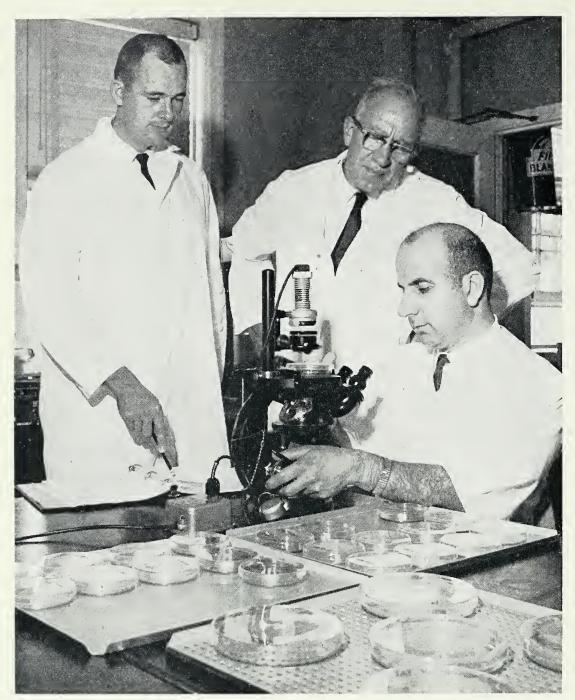
ARS plant physiologist Max W. Williams, cooperating with the Washington Agricultural Experiment Station, Wenatchee, used Red Delicious apple trees 4 to 5 feet tall on seedling rootstock, dug from the nursery in the fall, and held in storage at temperatures of 35° to 40° F. One and two months before planting, the cytokinins, BA and BAP, and the gibberellins, GA<sub>4</sub> and GA<sub>7</sub>, were applied in a lanolin paste by brush to the dormant buds and portions of the trunk around the buds. After planting and when the

shoots from the treated buds were about an inch long, all the trees were topped, or headed.

Six to seven months later when Dr. Williams measured the results, BA and BAP alone or in combination with GA<sub>4</sub> and GA<sub>7</sub> had significantly increased the number of buds breaking dormancy and almost doubled the number of growing limbs, when compared to the control trees. GA<sub>4</sub> and GA<sub>7</sub> used alone had no significant effect. Although both BA and BAP significantly increased crotch angles, BAP was more effective.

The usual methods of increasing the number and crotch angles of shoots entail various cultural practices such as notching above buds and low heading, and mechanical methods of spreading to increase the crotch angles. Although these methods are helpful, they often do not entirely achieve the desired goals.

The major benefit to be derived from bud treatments is getting the trees off to the best possible start with a great variety of more horizontal limbs in many positions around the trunk for a wider choice when pruning. Thus with the growth regulators, growers can form better trees earlier.



Veterinarian Richard L. Witter, biologist Ben R. Burmester, and microbiologist John J. Solomon, all of ARS, examining cell cultures for evidence of infection with Marek's disease (672232-1).

# Do Eggs Transmit Marek's The answer seems to be no, based on strong circumstantial evidence obtained in current ARS-

Michigan studies.

This research suggests the feasibility of control or eradication by rearing chicks from infected breeder flocks in rigidly controlled isolation. Chicks free of infection at hatching should remain free if produced under

conditions that preclude exposure to the virus. Marek's disease (MD) increases the cost of producing and processing poultry and eggs by more than \$200 million annually.

Scientists at the ARS Regional Poultry Research Laboratory, East Lansing, Mich., say research results to date do not support an unequivocal statement that embryo transmission of MD herpesvirus does not occur. They note possible limitations in the sensitivity of their tests and sampling procedures. And they point out no tests have been made with eggs containing blood or meat spots or with eggs from recently infected hens that have not yet developed antibody.

Nevertheless, ARS microbiologist John J. Solomon says, "The data clearly establish that this source of infection is not of sufficient importance to preclude either the production of infection-free flocks by isolation rearing or the consideration of this procedure for the control and possible eradication of this disease."

ARS veterinarian Richard L. Witter, ARS geneticist Howard A. Stone, and geneticist Lloyd R. Champion of the Michigan Agricultural Experiment Station also participated in the research.

The scientists isolated no MD virus from tissues of 1,180 embryos or newly hatched chicks from nine infected breeder flocks. And 1,484 chicks from these flocks were free of infection at hatching, as determined by absence of MD antibody after 9 or more weeks of isolation rearing.

Evidence against embryo transmission of MD was presented by scientists as long ago as 1951, although it was not confirmed by some recent studies. Dr. Solomon and his colleagues therefore attempted to demonstrate MD herpesvirus both directly and indirectly in the progeny of naturally infected flocks, using the most sensitive techniques currently available.

In one series of experiments, they used techniques that would indicate the presence of MD herpesvirus in embryos or newly hatched chicks. Two cell-culture methods, bioassay in chicks, and direct examination of embryos were used.

In addition, the scientists reared chicks in two types of isolation chambers, then tested for infection after 9 to 26 weeks. Evidence of embryo transmission was sought by testing for presence of the antibody and by histopathological examination.

Neither the virus isolation nor the isolation rearing studies indicated that MD herpesvirus is transmitted via the embryo. Nerve lesions, which the scientists believe are unrelated to MD herpesvirus, were detected in 13 of more than 1,300 microscopic examinations.

All of the source flocks used in the study were known to carry high levels of infectivity. In Flock A, for example 13 to 28 percent tested positive for MD virus in the blood and 71 to 81 percent showed the antibody during the period when the egg transmission tests were made. Total MD mortality in this flock was 33 percent through 76 weeks of age.

Seven Laboratory or Station breeding flocks used as egg sources included lines known to be high, intermediate, and low in relative susceptibility to development of MD lesions. Two commercial flocks in Georgia also provided eggs.

In related research, eight flocks of about 2,900 chickens from presumably infected sources in Georgia, New Hampshire, Indiana, and Iowa were found free of MD infection, as determined by absence of the antibody in 10 or more blood serum samples obtained from each.



THE LONDON PLANE has proved extremely valuable as a tree that can endure the environmental hardships of modern cities.

The first London plane, a cross between the oriental plane and the American plane, was a hybrid of accidental origin. However, it is doubtful whether any living, mature tree today could be verified as a true first-generation hybrid.

The London planes on our city streets today are a complex mixture of many generations of crosses between hybrids and between hybrids and parents. Not only do these trees exhibit wide variation in growth characteristics, but they vary in their tolerance to air pollution and resistance to sycamore anthracnose disease.

In the 300 or so years since the tree's origin, it is noteworthy that no attempts have been made to cross the American and Oriental species to achieve a true first-generation hybrid.

As part of an extensive breeding program to develop better trees for city planting, ARS geneticist Frank S. Santamour, Jr., made reciprocal crosses between 14-year-old oriental planes of authentic Turkish origin and mature native American planes growing on the grounds of the National Arboretum in Washington, D.C.

Seed from these crosses were planted along with non-hybrid seed from the two species in April 1969. All received the same cultural treatment.

When height measurements were taken in October 1969, unusual vigor was apparent in the hybrids. The average height of the hybrids was nearly twice that of the parent species.

Dr. Santamour is continuing his observations to determine whether or not this juvenile superiority will be maintained as the trees grow and mature.



# the Wheat We Eat

READ has been called the "staff of life" throughout the centuries, but so far no one knows exactly what it contributes to the body.

Our knowledge of the nutrient content of flour, bread, and the other wheat products is incomplete even though they provide about 15 percent of the protein, 16 percent of the calories, 29 percent of the carbohydrate, and 20 percent of the iron in U.S. diets. A recent ARS diet survey shows increased use of bakery products, but the nutrient composition of these foods has received little attention.

Now ARS scientists cooperating with colleagues at the American Institute of Baking and the Purdue Research Foundation have studied the changes in nutritive value produced by milling and baking while trying to determine the contribution of wheat products to the American diet.

Previous wheat studies determined the value of one nutrient at a time with results that were difficult to assemble into a single composition table. The ARS-sponsored research included an across-the-board check of all nutrients in a particular sample.

In a six-part study the three groups of scientists analyzed the composition of 10 types of wheat food products available to consumers living in two cities in each of five geographical regions of the United States and of

wheat grains and flours from these grains. Typical products made from these flours included all-purpose flour, biscuit mix, enriched white breads (continuous-mix and conventional sponge-dough procedures), whole wheat bread, macaroni, cake, crackers, hamburger rolls, doughnuts, and several types of breakfast cereals.

Wheat for these products came from five different growing areas and included hard red winter and spring wheats, soft red winter wheat, soft white wheat, and durum wheat.

Grains, flours and wheat products were examined for moisture, ash, protein, 18 commonly occurring amino acids, 15 fatty acids, and 5 carbohydrates. Four B-vitamins were also included along with different forms of vitamin E. Several minerals were also studied.

First results concern vitamin E and the B-vitamins.

Processing into white flour removed a major part of the vitamin E components called tocopherols. About 10 percent survived milling and bleaching in the soft and hard wheats compared to about 40 percent in the durum wheat after milling alone. This reflects the difference in the distribution and make-up of the durum wheat—which has a different genetic background—and the fact that the Semolina flour milled from the durum wheat was not bleached. A further loss

results when the Semolina is made into macaroni.

The study showed that vitamin E is particularly sensitive to bleaching treatment. The soft and hard wheat flours before bleaching retained almost the same amount of tocopherols as did the unbleached Semolina flour from the durum wheat. The further loss is due to bleaching. Vitamin E in the baked products made with vegetable shortening came mainly from the shortening.

Major losses also occurred in the B vitamins—thiamin, riboflavin, niacin, and vitamin  $B_6$ —during processing to flour. The vitamins retained in hard and soft wheats were directly related to the yield of flour from milling with less than 20 percent remaining at a 60 percent yield. Vitamin  $B_6$  was affected the most.

Hard and soft wheat flours retained about 25 percent of the thiamin and riboflavin of the whole wheat grain while flour from durum wheat retained about 50 percent of the thiamin and almost all of the riboflavin. Less than 15 percent of the vitamin B<sub>6</sub> of whole wheat was found in bread, 7 percent in cake, 10 to 20 percent in crackers, and 25 percent in macaroni made from durum wheat.

Nutrients in baked or otherwise processed products reflected the small amounts retained in the flour after milling rather than losses during baking, except where recipe ingredients contributed to the vitamin content.

In summary, durum wheat retained a larger proportion of its nutrients than did the hard and soft wheats. Treatments such as bleaching caused greater losses than the milling process for some nutrients. Wheats grown in different areas of the United States showed no significant difference in nutrient value after processing.

## AGRISEARCH NOTES

## Latest on Oral Insecticides

Tiny capsules enclosing an oral insecticide for dairy cattle greatly improve insecticide's efficiency in fly control.

Oral insecticides could eliminate much of the need for sprays, dusts and baits now used to control flies and maintain health and sanitation standards in milk production. Fed in the concentrate portion of the cow's diet, an oral insecticide passes through the digestive system and out in the feces where it kills fly larvae as they hatch.

When ARS scientists fed 2-chlorol-(2,4,5,-trichlorophenyl) vinyl dimethyl phosphate (Gardona) in capsule form to dairy cows, approximately 30 times more of the insecticide was found in the feces than when it was fed as a powder.

The tiny capsules, individually too small to be seen by the naked eye, allow more of the insecticide to pass through the digestive system and restrict it from being metabolized. The portion of the insecticide which is metabolized, however, is broken down into apparently harmless metabolites.

This research is being conducted at Beltsville. Md., by dairy husbandman Richard W. Miller. nutritionist Chester H. Gordon, chemist Morton Beroza, and entomologist Neal O. Morgan, and in Tifton, Ga., by chemist Malcolm C. Bowman.

Though only in the experimental stage, this oral insecticide shows great promise as a future means of house fly control. In field tests, 94 percent of fly larvae were killed when 64 p.p.m. insecticide in the encapsulated form were fed to cattle daily for a 3-week period. This appeared to be the most effective experimental dosage.

Residue studies showed that no insecticide appeared in the milk even when levels as high as 108 p.p.m. were fed to cows. And, concentrations a thousand times greater than those needed to kill fly larvae did not harm livestock or the person working with the insecticide.

This pesticide is for experimental use only and has not been registered by USDA for this purpose.

## Maturing Citrus in Storage

A procedure for maturing citrus fruits after harvest is under development to even out the peak and slack periods of citrus processing.

The experimental process involves storing the fruit for about 24 hours in a warm oxygen-free atmosphere of nitrogen or carbon dioxide. This results in mature fruit with decreased acid and increased sweetness. ARS chemists Joseph H. Bruemmer and Bongwoo Roe are developing the process at the U.S. Fruit and Vegetable Products Laboratory, Winter Haven, Fla.

The scientists are essentially mimicking nature. The fruit, as it matures naturally on the tree, lowers its oxygen demand to allow certain metabolic processes to take place that reduce acid content and thereby increase sweetness.

The process is not yet ready for commercial application. The scientists believe there may be other gases that will start and stop specific metabolic changes and result in perfectly matured fruit.

## UNITED STATES GOVERNMENT PRINTING OFFICE DIVISION OF PUBLIC DOCUMENTS, WASHINGTON, D.C. 20402

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United States Department of Agriculture

## AGRISEARCH NOTES

## Rx for Cloud Loss

Scientists have invented a new process to stabilize cloud and prevent orange juice from becoming clear in storage.

The new process involves the use of a pectin-degrading enzyme and could result in the elimination of the current practice of stabilizing the cloud with head which often causes undesirable flavor changes. Research on the development is being conducted by ARS chemists Robert A. Baker and Joseph H. Bruemmer at the U.S. Fruit and Vegetable Laboratory, Winter Haven, Fla.

Current practice in the citrus processing industry calls for heating orange juice to 190° F. to stabilize the cloud. In this treatment, heat inactivates pectinesterase, an enzyme naturally present in orange juice. If left active, the enzyme does not completely break down soluble pectin, but acts on it just enough so that it is subject to flocculation and settling. When the floc settles to the bottom of the container, it pulls the cloud down with it, leaving an unsightly clear liquid at the top and a coagulated mass forming at the bottom.

The phenomenon, known to the trade as cloud loss, has been a stimu-

lus for much research on the pectic substances and pectic enzymes in citrus and tomato products. Recent studies indicated that pectin is not necessary for cloud stability. Based on those studies Mr. Baker and Dr. Bruemmer decided to try to hydrolyze or break down the soluble pectin completely to galacturonic acid, a harmless sugar derivative that would not change the flavor of the juice.

Theoretically stabilizing the cloud with enzymes would permit juice processing without heating. The enzymes could be used in conjunction with a cold sterilant such as diethylpyrocarbonate and eliminate even the relatively mild temperatures (150°–160° F.) used to pasteurize the juice.

CAUTION: In using pesticides discussed in this publication, follow directions and heed precautions on pesticide labels. Be particularly



careful where there is danger to wildlife or possible contamination of water supplies.

## Rabbits Carry Nematodes to Sheep

Black-tailed jackrabbits may be infecting domestic range sheep with a common intestinal nematode.

ARS parasitologist Kay S. Samson of Las Cruces, N. Mex., has discovered that a nematode, *Trichostrongylus colubriformis*, common to range sheep in the Southwest, is also found in jackrabbits.

To test for possible transmission from rabbit to sheep, Dr. Samson collected nematodes from five jackrabbits. After removing eggs from *Trichostrongylus* females and culturing the eggs in feces of worm-free sheep, he fed 100 of the larvae to a 5-month-old lamb. He also cultured intestinal contents of jackrabbits and gave 4,000 of the mixed larvae he recovered to two lambs.

After about 3 weeks, nematode eggs began to appear in the feces of the lambs fed the larvae. Adult *T. colubriformis* were found in the small intestines when the lambs were slaughtered.

Dr. Samson thus demonstrated that natural infections of *T. colubriformis* in jackrabbits are transmissible to range sheep, and he believes the jackrabbit may be an important reservoir of infection.